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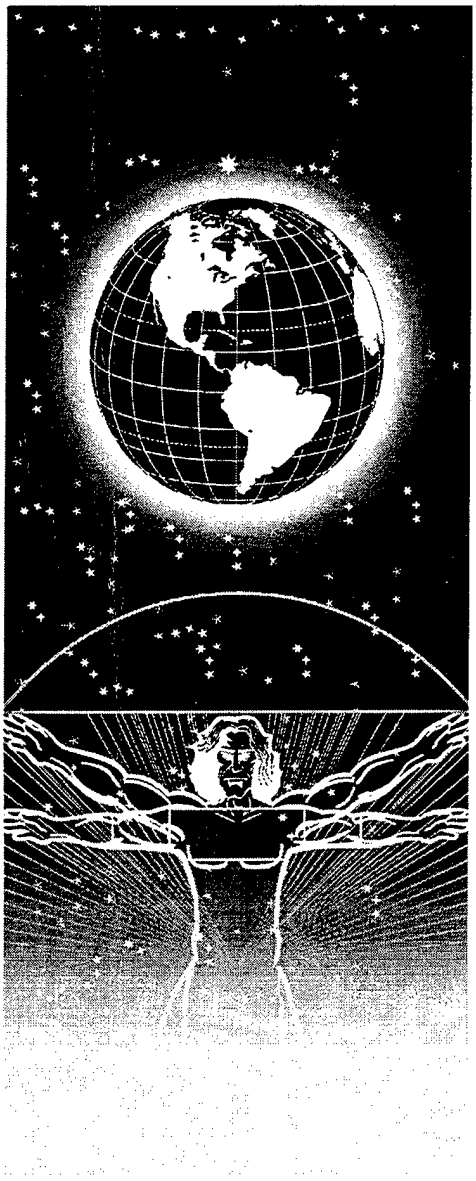

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

Acute and Subchronic Toxicity Evaluations of the Halon Replacement Candidate Phosphorus Tribromide

Robin E. Wolfe
Marcia L. Feldmann
David H. Ellis
Harry F. Leahy
Carlyle D. Flemming
Darol E. Dodd

MANTECH - GEO-CENTERS JOINT VENTURE
TOXIC HAZARDS RESEARCH
P. O. BOX 31009
DAYTON OH 45437-0009

Jeffrey S. Eggers

ARMY MEDICAL RESEARCH UNIT
WRIGHT-PATTERSON AFB OH 45433-7400

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Occupational and Environmental Health Directorate
Toxicology Division
2856 G Street
Wright-Patterson AFB OH 45433-7400

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This technical report has been reviewed and is approved for publication.

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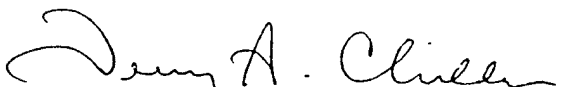

TERRY A. CHILDRESS, Lt Col, USAF, BSC
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Armstrong Laboratory

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Phosphorus tribromide (PBr_3) is being considered by the DOD as a possible replacement for Halon 1301. This study was designed to determine the effects following single, high-concentration exposures which could occur in accidents, as well as repeated, low-concentration exposures which could occur on flight lines or where maintenance commonly occurs. Application of 10 or 50 µL neat PBr_3 to intact skin of an anesthetized NZW rabbit caused edema and necrosis of the treated skin within 10 minutes of dosing. Microscopic examination confirmed necrosis of the skin and underlying areas, including the skeletal muscle of the subcutis. Acute 4-h nose-only exposure of Fischer 344 rats to PBr_3 vapor resulted in mortality at 4.1 mg/L. At 1.5 mg/L, labored breathing, body weight loss, ulceration of anterior nares, and rhinitis of the nasal passage were observed. Toxicity was not observed in rats exposed for 4 h to 0.4 mg/L. Male rats (5/group) were exposed to PBr_3 vapor, 4 h/d for 5 d, at 0, 0.06, 0.16, and 0.51 mg/L PBr_3. There were no signs of toxic stress. Rats of the 0.51 mg/L group had decreased body weights; gross lesions (reddened nares) and microscopic lesions (inflammation of mucosa and ulceration of epithelium of the nares) were also observed. Rats (10/sex/group) were exposed to PBr_3 vapor, 4 h/d, 5 d/wk, for 4 wk at 0, 0.03, 0.1, and 0.3 mg/L. There were no signs of toxic stress, alterations in body weights, or changes in organ weights in PBr_3 exposed animals. Minor serum chemistry and hematology effects were observed in the treated animals. Microscopic tissue findings were limited to rats of the 0.3 mg/L group and consisted of mild inflammation of the nasal passages. A concentration of 0.1 mg/L is the no observable adverse effect level (NOAEL) in the 28-day inhalation study.

14. SUBJECT TERMS
Phosphorus tribromide, PBr_3, ozone depleting substance replacement candidate, acute toxicity, subchronic toxicity, limit test, nose-only inhalation, repeated exposure, skin irritation, Fischer 344 rats, NZW rabbits

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxicology Division under the ManTech Geo-Centers Joint Venture contract. This document serves as a final report on the acute and subchronic toxicity evaluation of the halon replacement candidate phosphorus tribromide. The research described in this report began in October 1996 and was completed in March 1997 under Department of the Air Force Contract No. F41624-96-C-9010. Lt Col Terry A. Childress served as the Contracting Officer's Representative for the U.S. Air Force, Armstrong Laboratory. Darol E. Dodd, Ph.D., served as Program Manager for ManTech Geo-Centers Joint Venture.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended. The authors gratefully acknowledge the excellent technical assistance of Richard J. Godfrey, Jerry W. Nicholson, and Margaret A. Parish.

ABBREVIATIONS

Br_2	Bromine
°C	Degrees centigrade
dL	Deciliter
EC_{50}	Median effective concentration
°F	Degrees Fahrenheit
F-344	Fisher 344 (rats)
g	Gram(s)
h	Hour(s)
HBr	Hydrogen bromide gas
IU	International Units
kg	Kilogram
L	Liter
LC_{50}	Median lethal concentration
mg	Milligram
min	Minutes (s)
mL	Milliliter
mmol	Millimoles
N	Number
NOAEL	No observable adverse effect level
NZW	New Zealand white (rabbits)
PBr_3	Phosphorus tribromide
PPM	Parts per million
SD	Standard deviation
µL	Microliter(s)
µM	Micromolar

SECTION I

INTRODUCTION

The Department of Defense requires the development of a complete toxicity profile for replacement candidates of halons, which includes the results from acute and subchronic toxicity testing. Many of these compounds, including phosphorus tribromide, have not been thoroughly investigated to determine their toxicological properties. The purpose of this study was to conduct acute and subchronic toxicity testing to provide acute toxicological hazard information to complete a health hazard assessment for phosphorus tribromide. The health hazard assessment will help determine if phosphorus tribromide (PBr_3) can be used as a Halon replacement compound. U.S. Air Force tests in a 500,000 BTU/h test burner demonstrated that PBr_3 is an effective fire extinguishant. The test burner was quenched by only 0.2 mL PBr_3. This volume is several thousand times less than the amounts required of other halon replacements to suppress fires. The new fire extinguishant systems based on PBr_3 usage will occupy less volume, weigh less, and require less mechanics than current systems used on U.S. Air Force aircraft and electronic equipment fires. Also, PBr_3 has no ozone depleting potential since it is rapidly hydrolyzed in the troposphere.

Phosphorus tribromide reacts with moisture in the air to produce phosphonic acid and hydrogen bromide gas (HBr). There is limited toxicity information available in the literature on phosphorus tribromide and hydrogen bromide. No acute toxicity information is available for phosphonic acid or hydrobromic acid, the aqueous form of HBr. The combined interaction of these compounds to cause potential health hazards is unknown.

An accidental human exposure to a mixture of PBr_3 and hydrogen bromide was reported by Kraut and Lilis, 1988. While mixing PBr_3 and hydrogen bromide, a female laboratory assistant was exposed to these compounds via splashing on the face, chest, and hair, and by inhalation of resulting vapors. She remained in the area of the exposure for five to ten minutes before receiving treatment for the chemical burn. Immediate effects noted were dry cough, light-headedness, and slight congestion of the throat. Over the next two weeks, the subject experienced increasing shortness of breath. Chest x-rays revealed bilateral lobe infiltrates, and chemical pneumonitis was diagnosed. She was allowed to return to work a few months later, though dyspnea on exertion persisted and chest x-ray findings had not yet completely resolved.

No exposure assessment was conducted and attempts to get exposure information was not successful.

Tissue injury of the respiratory tract was observed in a study of rats exposed to 1300 ppm HBr for 30 min that compared nose-breathing effects to pseudo-mouth-breathing effects (Stavert et al., 1991). Tissue damage following nose-breathing exposure to HBr was confined to the nasal region. Observations included epithelial and submucosal necrosis. Pseudo-mouth-breathing exposure to HBr caused higher mortality rates and major tissue disruption was noted in the trachea. Observations included epithelial, submucosal, grandular, and cartilage necrosis.

Results of genotoxicity testing of bacterial Salmonella strains (ManTech, 1996) indicated PBr_3 is not a mutagen for both frame shift and base-pair substitution tester strains in both buffered and unbuffered solutions. Acute aquatic toxicity tests on the fathead minnow and *Daphnia magna* have also been conducted with PBr_3. The 96-h LC_{50} value for fathead minnow was 71 mg/L (50 - 100 mg/L confidence limit); the no observed effect concentration was 25 mg/L (Aqua Survey, 1996a). The 48-h EC_{50} value for *Daphnia magna* was 22.6 mg/L (18.2 - 27.9 mg/L confidence limit); the no observed effect concentration was 6.25 mg/L (Aqua Survey, 1996b).

The objectives of this study were to determine the acute and subchronic toxicity of PBr_3. The information gathered from these experiments should provide a preliminary database for developing a health hazard assessment for PBr_3.

SECTION II

MATERIALS AND METHODS

Test Material

Phosphorus tribromide (PBr_3)

Source/Manufacturer:	Aldrich Chemical Company, Milwaukee, WI
CAS No.:	7789-60-8
Purity:	Certified by the manufacturer as 99.99+% pure
Appearance:	Colorless to pale yellow liquid
Specific Gravity:	2.850 g/mL
Vapor pressure:	0.27 psi at 54 °C

Test Animals

Male and female Fischer-344 (CDF®[F-344]/CrlBR) rats weighing between 100 and 125, and 75 and 100 g, respectively, were purchased from Charles River Laboratories. Male New Zealand white (NZW) rabbits weighing between 2 and 3 kg were purchased from Myrtle's Rabbitry, Inc. All animals were subjected to a two-week quarantine period. The animals were housed in laminar-flow rooms during nonexposure periods. Rats were group housed (two per cage) in clear plastic cages with hardwood-chip bedding (SANI-CHIPS®, P.J. Murphy Forest Products, Montville, NJ). The rabbits were housed individually in wire-bottom, stainless-steel cages. Water and feed (Purina Rabbit Chow #5320, and Purina Certified Rodent Diet #5002) were available *ad libitum*, except during the inhalation exposure period, and for 12 h prior to sacrifice for the 5-day range-finding rats only. Animal room temperatures were maintained at 21 to 25 °C and the light/dark cycle was set at 12-h intervals. Plexiglas restraining tubes used with the nose-only chambers were cleaned as needed to provide enough clean tubes for each day's exposure.

Experimental Design

Acute Toxicity Tests

The approach for determining the acute toxicity of PBr_3 was to perform a skin irritation screen using rabbits and an acute inhalation test using rats. The information gathered in these tests will provide useful information necessary to establish limits for emergency conditions resulting from spills or leaks involving PBr_3.

Skin Irritation Screen

Due to the potential corrosiveness of PBr_3, a skin irritation screen was performed to establish if a minimally irritating volume of PBr_3 could be determined. Two male New Zealand white (NZW) rabbits had hair shaved from their backs 24-h prior to the Skin Irritation Screen. The first rabbit was anesthetized with xylazine hydrochloride (9 mg/kg) plus atropine (0.16 mg), and ketamine hydrochloride (40 mg/kg) administered IM. Once the animal was anesthetized, a neat dose of 50μL (0.050 mL) test material was applied to a designated area of the shaved back. The test material was allowed to react with the skin for 10 minutes before observations were made. The skin was then observed and any irritation recorded (Draize, 1959). Since irritation occurred at the initial 50 μL dose, a PBr_3 dose volume of 10 μL was applied to an area of the shaved back adjacent to the 50 μL dose, and allowed to remain for 10 minutes before observations were made. Any signs of irritation were recorded. Since irritation was apparent at the 10 μL dose volume, the PBr_3 test material was determined to be too irritating to the skin to perform a complete 72-h skin irritation test.

A second rabbit was also anesthetized, as above, and a neat dose of 50 μL PBr_3 was applied to a designated area of the shaved back. The treated skin was then washed for one minute with copious amounts of water 30 seconds after dosing. The skin was then observed 10

minutes after flushing, and any signs of irritation were recorded (Draize, 1959). Since irritation was observed, this procedure was repeated using a PBr_3 dose volume of 10μL.

The two rabbits utilized in the skin irritation screen were euthanatized at the conclusion of testing, while still under anesthesia, with a percutaneous intravenous injection of Euthol euthanasia solution.

Acute Nose-Only Inhalation Exposure

Five female Fischer 344 (F-344) rats were exposed to PBr_3 for four hours duration at the limit test value of 5 mg/L (USEPA, 1990). A series of acute nose-only inhalation exposures were then performed due to deaths or signs of toxic stress observed during the limit test. Four additional exposures were carried out, spaced to produce a range of toxic effects. Male F-344 rats were used at concentration levels below the limit test. Five male rats per group were exposed to target PBr_3 concentrations of 2.5, 1.0, and 0.5 mg/L. All animals were weighed prior to exposure and on postexposure days 1, 2, 7, and 14. Exposed animals were observed for signs of toxic stress twice daily. All animals received a gross necropsy at sacrifice. Animals were euthanatized via CO_2 inhalation. Lungs from animals dying immediately postexposure were taken for histopathologic examination. Exposures were performed using a nose-only Cannon-52 exposure chamber (Cannon et al., 1983).

Generation and Analysis. The required concentration of phosphorous tribromide was generated using Sage syringe pumps delivering the required mass of test material into the air supply for the Cannon-52 chambers. The Cannon chamber was housed within a 690-L inhalation exposure chamber, which was used as a containment area to isolate the exposure from the general laboratory. A minimum of 300 to 500 mL/min of dry (<3% RH) filtered house air was supplied per animal in the test system with a minimum of 10 L/min used for each of the test groups. The amount of test material delivered per unit time was calculated on the basis of air flow and desired concentration. PBr_3 is unstable in the presence of water vapor; therefore the test material analysis required quantitating the sample prior to mixing with expired air. Analysis for bromide ion (Bromide specific ion electrode, Model 463500, Orion Research, Inc.,

Cambidge, MA) was performed to determine PBr_3 test material concentration. A grab sample technique was employed to collect material for bromide ion analysis.

Subchronic Toxicity Tests

Five-Day Inhalation Concentration Range-Finding Study

A one-week (5 exposures) pilot study was performed to determine target concentration levels for a subchronic 28-day study. Three concentrations of PBr_3, 0.5, 0.1, and 0.05 mg/L, were used in this concentration range-finding study. A control group was also included. The highest concentration of PBr_3 was determined considering the results of the acute inhalation toxicity test. Five male F-344 rats were exposed via nose-only inhalation to air only or PBr_3 for 4 h/day for 5 consecutive days. Exposures were performed using Cannon chambers. Animal body weights were measured pre-exposure and each exposure day. Animals were fasted 12 h prior to necropsy and terminal body weight were also recorded. A clinical examination was performed for each animal every study day. Animals were observed daily for visible signs of toxicity, and all observations were recorded. Animals were euthanized via CO_2 inhalation. Blood was collected at necropsy via the posterior vena cava for complete clinical chemistry and hematology evaluations. At necropsy wet tissue weights were determined for heart, kidneys, testes, liver, lungs, brain, spleen, thymus, and adrenal glands. A gross pathologic examination was performed on each rat. The upper respiratory tract and gross lesions were taken for histopathologic examination.

Generation and Analysis. Details are given in the "Subchronic Inhalation Toxicity Study (28 Days)" section described below. For personnel safety, the Cannon 52 units were operated within a set of Plexiglas boxes, and were vented through a vacuum pump which delivered the PBr_3 vapor to a water scrubber exhaust system. The containment areas were further isolated from the general laboratory area through use of a separate exhaust line which vented the enclosures.

Subchronic Inhalation Toxicity Study (28 Days)

The concentrations of PBr_3 utilized in the 28-day inhalation study were 0.3, 0.1, 0.03, and 0.0 (air control) mg/L. These concentrations were determined upon completion of the data analyses from the 5-day range-finding study. Ten male and 10 female F-344 rats per group were exposed to PBr_3 or air only for 4 h/day, excluding weekends, for 28 days (a total of 20 exposures). Animal body weights were measured prior to the initial exposure and then weekly thereafter. A clinical examination of each animal was made on each exposure day. The animals' general health condition and any clinical signs of toxic stress were recorded. The animals were fasted 12 h prior to necropsy. A complete blood assay was conducted on blood samples taken at sacrifice from the posterior vena cava of all animals. The animals were euthanatized via CO_2 inhalation. At necropsy wet tissue weights were determined on adrenal glands, lungs, brain, ovaries or testes, heart, spleen, kidneys, thymus, and liver. A gross pathologic examination was performed on each rat. Full histopathology was performed on organs and tissues listed below for all animals in the control and high-concentration exposure groups. These tissues were taken from the low- and mid-concentration groups and held for examination if target organs or tissues were identified in the high-concentration animals. All gross lesions were taken for histopathologic examination.

TISSUES COLLECTED FOR HISTOPATHOLOGIC EXAMINATION FROM F-344 RATS FOLLOWING REPEATED EXPOSURE TO PBr_3

Brain	Lungs	Prostate
Pituitary	Trachea	Epididymides
Spinal cord	Nasal turbinates	Testes
Sciatic nerve	Esophagus	Ovaries
Heart	Stomach	Uterus
Liver	Duodenum	Urinary bladder
Kidneys	Jejunum	Salivary glands
Adrenal glands	Ileum	Mandibular lymph nodes
Pancreas	Cecum	Mesenteric lymph nodes
Thyroid/parathyroid	Colon	Sternum w/ bone marrow
Thymus	Rectum	Bone (femur including stifle)
Spleen	Skeletal muscle (thigh)	

Generation. Four Cannon nose-only inhalation chambers were supplied with dried laboratory air with a minimal air flow delivery rate of 500 mL/animal. The air dryer reduced the relative humidity to <3% as measured by a HY-CAL® detector (Model CT-830-D, HY-CAL Engineering, El Monte, CA). This detector also monitored the temperature of the carrier air. The generation system was similar to that used in the 5-day study. Gas tight syringes (1.0, 0.5, and 0.25 mL) were used for direct delivery (needle tip evaporation) into the 10 L/min dried air stream. Sage® syringe pumps (Model 355, Orion Research, Inc., Cambridge, MA) were used to control the input of the test material. A bypass and containment dump system was employed to allow for smooth initiation of exposures and for use as a safety measure in case of abnormal syringe pump operation. The bypass air input was used both before and after the 4-h exposures to supply clean air, and was used along with the PBr_3 input carrier air to control the concentration of the exposure atmospheres.

Analysis. Details of the analytical method and approach are given in Appendix A. A Bromide specific ion electrode (Model 463500, Orion Research, Inc., Cambridge, MA) permitted quantification of the test material concentration through analysis of the bromide ion absorbed in an ionic strength buffer (pH 4.0). In order to monitor the lower concentrations, the ratio of vapor sample to buffer had to be increased from 10:1 air/absorber to 20:1 and 40:1. This allowed operation in the linear portion of the response curve. Sixty-mL plastic syringes containing 5.0 mL buffer were used to quantify the 50 mL vapor samples and acted as the reaction vessel for the absorption of PBr_3. Multiple absorption samples were required to analyze the lower chamber concentrations.

Statistics

Body weights were analyzed using the repeated multivariate analysis of variance with Scheffe pairwise comparisons (Barcikowski, 1983). Hematology, clinical chemistry, and organ weights were analyzed using a two-factorial analysis of variance with multivariate comparisons (Barcikowski, 1983). Histopathology data were analyzed through use of the Fischer Exact Test, or, if not valid, Yates' Corrected Chi-square (Zar, 1974).

SECTION III

RESULTS

Acute Toxicity Tests

Skin Irritation Screen

Application of 50 μL or 10 μL neat PBr_3 to intact rabbit skin caused an immediate reaction, producing a white vapor. No immediate change in the color of the rabbit skin occurred, though the treated areas were hardened when compared to adjacent untreated skin. Ten minutes after dosing, the skin of both rabbits (where site of test substance application was either rinsed with water or unrinsed) had severe edema and necrosis for each volume (50 or 10 μL) of PBr_3 used. The affected skin was limited to the treated area only and was clearly demarcated from the adjacent untreated skin.

The animal tested dermally with no rinsing of test material had both skin and muscle lesions noted at necropsy. These tissues were collected and examined histologically. Findings included focally extensive necrosis of the entire thickness of the haired skin, epidermis, and dermis. Focally extensive and severe hyaline degeneration and necrosis of the panniculus muscle were observed. Hyaline degeneration and necrosis were also observed in the skeletal muscle of the underlying subcutis. In the second rabbit tested dermally, but with the test material rinsed with water from the skin, only skin lesions were noted at necropsy. The skin lesion was collected and examined histologically. As in the first rabbit, histologic findings included extensive necrosis of the entire thickness of the haired skin, epidermis, and dermis. Multifocal, moderate hyaline degeneration and necrosis of the panniculus muscle were also noted. Injury did not spread to the underlying subcutis. No further testing for skin irritation was performed.

Acute Nose-Only Inhalation Exposure

Table 1 contains body weights and mortality information for the acute inhalation exposures. One of five female rats from the 4.1 mg/L PBr_3 nose-only inhalation exposure died within five min of the conclusion of exposure. Another female was found dead and the other three females were euthanized as moribund one day postexposure. Observations during exposure included labored breathing and mouth breathing. During the first hour of exposure, all animals tried to avoid breathing the exposure atmosphere by attempting to withdraw their noses and heads from the nose cone of the exposure tubes. At necropsy, gross findings consisted of 4 of 5 animals with nares plugged with a black substance, and 1 of 5 animals had epidermal layer of nares missing. Histologic findings consisted of severe, diffuse, peracute necrosis of the lamina propria epithelium of the nasal passages, and moderate to severe, multifocal, acute, necrosis of the turbinate epithelium.

Animals in the 1.5 mg/L exposure group showed signs of labored breathing and some struggled to pull away from the PBr_3 atmosphere during exposure. Postexposure, the animals appeared mildly unsteady with labored breathing. Body weights were lower than expected for postexposure Days 1 through 7 (Table 1). All animals survived the 14-day observation period. Gross findings consisted of tips of nares missing and deep ulceration of the external nares with exposure of underlying cartilage. Histologic findings consisted of mild to moderate, suppurative to subacute, mulitfocal, rhinitis of the lamina propria of the nasal passages in all animals. One of five animals also displayed squamous cell metaplasia of the nasal passages (most anterior regions).

Observations of animals exposed to 0.9 mg/L of PBr_3 during the first hour included rapid breathing, pulling away from the PBr_3 air source to avoid the exposure atmosphere, twisting in the tubes and contracted abdominal area. Animals appeared less active after the first hour of exposure and displayed rapid shallow breathing. All animals survived the 14-day observation period, and there were no gross findings at necropsy.

Animals exposed to 0.4 mg/L PBr_3 appeared normal, and all survived the 14-day observation period. There were no gross findings at necropsy. Pathology findings were normal.

TABLE 1. DATA SUMMARY FOR THE ACUTE 4-h INHALATION EXPOSURES OF PHOSPHORUS TRIBROMIDE

Target Conc. mg/L	Nominal Conc. mg/L	Analyzed Conc. mg/L	Animal No.	Body Wt[a] Day 0	Body Wt Day 1	Body Wt Day 2	Body Wt Day 7	Body Wt Day 14
0.5	0.58	0.42	11	260.5	239.7	236.6	250.7	263.0
Males			12	252.9	234.2	229.9	243.1	258.1
			13	243.1	234.5	228.7	241.4	248.2
			14	232.7	225.9	223.3	236.0	224.0
			15	254.8	240.9	237.8	250.2	255.2
			Mean	248.8	235.0	231.3	244.3	249.7
			SD	11.0	5.9	6.0	6.2	15.3
1.0	1.07	0.89	06	256.6	247.8	241.4	251.6	260.9
Males			07	236.4	214.1	196.1	205.8	229.1
			08	232.6	215.2	200.1	210.1	236.3
			09	229.8	213.8	192.0	191.6	219.1
			10	248.1	236.0	225.7	242.1	254.0
			Mean	240.7	225.4	211.1	220.2	239.9
			SD	11.3	15.7	21.5	25.5	17.3
2.5	2.31	1.48	16	257.2	231.7	224.0	222.1	240.4
Males			17	255.8	229.8	214.0	198.2	234.6
			18	261.4	233.9	218.3	206.9	238.0
			19	267.0	240.4	224.3	197.8	219.4
			20	244.3	225.7	207.4	186.9	198.7
			Mean	257.1	232.3	217.6	202.4	226.2
			SD	8.4	5.4	7.1	13.1	17.4
5.0	4.97	4.09	21	168.0	156.2[b]			
Females			22	160.4[c]				
			23	161.5	150.8[b]			
			24	167.6	----[d]			
			25	166.7	157.5[b]			
			Mean	164.8	154.8			
			SD	3.6	3.6			

[a]Body weight in grams.
[b]Euthanized moribund postexposure day 1.
[c]Animal died immediately postexposure.
[d]Animal found dead postexposure day 1.

Subchronic Toxicity Tests

Five-Day Inhalation Concentration Range-Finding Study

Mean ± standard deviation exposure concentrations of PBr_3 were 0.06 ± 0.01,0.16 ± 0.03, and 0.51 ± 0.08 mg/L for target exposure concentrations of 0.05, 0.1 and 0.5 mg/L respectively. Analytical to nominal concentration ratios for these exposures were 0.4, 0.6, and 0.4, respectively. There were no deaths or signs of toxic stress during the 5-day exposure period. Mean body weights decreased in all exposure groups and were significantly different in the high concentration group when compared to control on Study Days 3 and 4 (Table 2).

TABLE 2. MEAN BODY WEIGHTS[a] OF MALE F-344 RATS DURING FIVE-DAY, NOSE-ONLY INHALATION EXPOSURE TO PHOSPHORUS TRIBROMIDE OR AIR

Day of Study	CONTROL	0.05 mg/L	0.1 mg/L	0.5 mg/L
0	258.2 ± 8.0	259.0 ± 10.4	255.6 ± 12.5	258.8 ± 10.7
1	250.4 ± 7.9	251.0 ± 10.5	245.3 ± 13.8	247.7 ± 12.6
2	245.4 ± 8.3	245.9 ± 9.4	241.6 ± 12.9	239.9 ± 10.2
3	242.7 ± 9.0	242.9 ± 9.8	237.3 ± 13.1	234.7 ± 9.9[b]
4	241.2 ± 9.0	240.5 ± 10.5	236.3 ± 12.0	229.0 ± 8.5[b]
5	225.7 ± 8.6	225.2 ± 8.6	220.6 ± 10.6	214.4 ± 7.9

[a]Mean ± SD, N=5.
[b]Significantly different from control at $p<0.01$.

Statistically significant differences between control and PBr_3- exposed animals were observed in several mean values of serum chemistry and hematologic parameters (Tables 3 and 4). Increases in serum chloride values were attributable to interference with the presence of serum bromide (See Discussion section). Increased values in calcium and potassium were noted in the 0.51 mg/L group. Decreased values when compared to control were observed in alkaline phosphatase and creatine kinase in the high-dose group; ALT values in the 0.16 mg/L and 0.51 mg/L groups. Additional statistically significant differences in mean values of serum

chemistry or hematologic parameters were sporadic and were not considered biologically important.

Gross observations at necropsy revealed irregular shaped and reddened nares in 3 of 5 animals in the 0.51 mg/L group. There were no gross lesions observed in the other exposure groups, including the control group. Absolute organ weight means and mean organ weight to body weight ratios (Table 5) revealed no statistically significant differences compared to control animals. Microscopic lesions were observed in the 0.51 mg/L group and in the anterior-most segment of the nasal passages. In this group, suppurative (acute) inflammation of the mucosa of the nasal passages occurred at a statistically higher incidence than control animals ($p<0.05$). Chronic ulceration of the epithelium of the external nares was observed in 3 of 5 animals. Minimal squamous metaplasia of the respiratory epithelium was observed in the trachea of one rat in the high concentration group. In the 0.16 mg/L PBr_3 group, 1 of 5 rats had slight inflammation of the nasal mucosa (most anterior regions). There were no microscopic lesions in rats of the 0.06 mg/L and control groups.

TABLE 3. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR MALE F-344 RATS AFTER FIVE-DAY, NOSE-ONLY INHALATION EXPOSURE TO PHOSPHORUS TRIBROMIDE

Parameter	Unit	Control	0.05 mg/L	0.1 mg/L	0.5 mg/L
BUN	(mg/dL)	15.8 ± 0.4	13.4 ± 1.3[b]	15.4 ± 1.1	16.6 ± 1.5
Creatinine	(mg/dL)	0.6 ± <0.1	0.6 ± 0.1	0.6 ± <0.1	0.6 ± <0.1
Chloride	(mmol/L)	95.0 ± 1.2	99.2 ± 3.5	106.0 ± 1.4[c]	130.4 ± 3.1[c]
Calcium	(mmol/L)	11.6 ± 0.3	11.4 ± 0.5	11.9 ± 0.4	12.4 ± 0.2[b]
Phosphorus	(mg/dL)	10.7 ± 0.6	10.3 ± 0.6	10.8 ± 0.6	10.7 ± 0.6
Total Protien	(g/dL)	6.6 ± 0.2	6.5 ± 0.3	6.5 ± 0.2	6.7 ± 0.2
AST	(IU/L)	97.6 ± 10.8	93.2 ± 11.6	84.2 ± 10.5	78.6 ± 6.0
ALT	(IU/L)	59.8 ± 5.1	55.0 ± 6.4	47.4 ± 6.1[b]	38.4 ± 3.7[c]
Alkaline Phosphatase	(IU/L)	151.0 ± 2.9	147.2 ± 12.1	139.0 ± 7.0	132.6 ± 8.4[b]
Glucose	(mg/dL)	179.8 ± 10.9	154.4 ± 16.8	179.4 ± 22.1	164.0 ± 14.9
Sodium	(mmol/L)	149.4 ± 1.1	150.8 ± 2.2	151.8 ± 1.3	151.8 ± 1.5
Triglycerides	(mg/dL)	102.6 ± 8.2	102.6 ± 43.9	93.6 ± 20.8	72.6 ± 22.1
Magnesium	(mg/dL)	2.4 ± 0.3	2.5 ± 0.5	2.6 ± 0.4	2.5 ± 0.3
Potassium	(mmol/L)	4.8 ± 0.3	4.8 ± 0.1	4.7 ± 0.1	5.4 ± 0.2[c]
Total Bilirubin	(mg/dL)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± <0.1	0.3 ± <0.1
ALB/GLOB	(g/dL)	1.2 ± <0.1	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
Uric Acid	(mg/dL)	1.6 ± 0.2	1.5 ± 0.2	1.8 ± 0.4	1.5 ± 0.5
Albumin	(g/dL)	3.6 ± 0.1	3.4 ± 0.2	3.6 ± 0.2	3.5 ± 0.1
Globulin	(g/dL)	3.0 ± 0.2	3.1 ± 0.2	2.9 ± 0.1	3.2 ± 0.1
Creatine Kinase	(IU/L)	69.0 ± 6.0	75.0 ± 16.3	59.8 ± 14.4	45.6 ± 10.7[b]
LDH	(IU/L)	246.2 ± 15.0	252.8 ± 19.4	267.0 ± 42.3	272.4 ± 63.7

[a]Mean ± SD, N = 5.
[b]Significantly different from control at p<0.05.
[c]Significantly different from control at p<0.01.

TABLE 4. BLOOD HEMATOLOGY VALUES[a] FOR MALE F-344 RATS AFTER FIVE-DAY, NOSE-ONLY INHALATION EXPOSURE TO PHOSPHORUS TRIBROMIDE

Parameter	Unit	Control	0.05 mg/L	0.1 mg/L	0.5 mg/L
WBC	(10^3)	9.6 ± 0.7	11.0 ± 1.3	9.9 ± 0.7	9.3 ± 0.9
RBC	(10^6)	9.7 ± 0.2	9.4 ± 0.2	9.7 ± 0.2	9.7 ± 0.2
HGB	(g/dL)	16.8 ± 1.0	16.2 ± 0.6	16.3 ± 0.7	16.2 ± 0.4
HCT	(%)	52.0 ± 1.0	50.8 ± 1.3	51.7 ± 1.0	52.6 ± 0.9
MCV	(g/dL)	53.7 ± 0.6	53.9 ± 0.2	53.2 ± 1.0	54.0 ± 0.2
MCH	(g/dL)	17.4 ± 0.8	17.2 ± 0.5	16.8 ± 0.7	16.7 ± 0.4
MCHC	(g/dL)	32.3 ± 1.6	31.8 ± 1.0	31.6 ± 1.0	30.8 ± 0.8
Platelets	(10^3)	938.0 ± 49.9	931.8 ± 49.2	957.8 ± 41.9	916.2 ± 22.8
Neutrophils	(%)	16.8 ± 2.2	15.1 ± 2.4	15.9 ± 3.2	20.2 ± 2.4
Lymphocytes	(%)	76.0 ± 2.7	83.2 ± 0.9	78.6 ± 5.5	72.4 ± 5.0
Monocytes	(%)	6.3 ± 0.8	1.2 ± 2.1[b]	4.4 ± 3.2	6.3 ± 3.5
Eosinophils	(%)	0.6 ± 0.4	0.5 ± 0.2	0.8 ± 0.2	0.8 ± 0.3
Basophils	(%)	0.4 ± 0.2	<0.1 ± <0.1[b]	0.2 ± 0.2	0.3 ± 0.2

[a]Mean ± SD, N = 5.
[b]Significantly different from control at $p<0.05$.

TABLE 5. ORGAN WEIGHTS[a] AND ORGAN-TO-BODY WEIGHT RATIOS OF MALE F-344 RATS AFTER FIVE-DAY, NOSE-ONLY INHALATION EXPOSURE TO PHOSPHORUS TRIBROMIDE

Organ	Control	0.05 mg/L	0.1 mg/L	0.5 mg/L
Body Wt	226 ± 8.6	225 ± 8.6	221 ± 10.6	214 ± 7.9
Liver	7.12 ± 0.72	6.88 ± 0.57	6.65 ± 0.37	6.47 ± 0.56
Ratio[b]	3.15 ± 0.21	3.05 ± 0.15	3.02 ± 0.13	3.01 ± 0.15
Kidneys	1.77 ± 0.08	1.76 ± 0.08	1.52 ± 0.41	1.68 ± 0.08
Ratio	0.78 ± 0.02	0.78 ± 0.02	0.69 ± 0.20	0.78 ± 0.02
Testes	2.84 ± 0.13	2.78 ± 0.09	2.53 ± 0.46	2.65 ± 0.12
Ratio	1.26 ± 0.02	1.24 ± 0.02	1.16 ± 0.24	1.24 ± 0.04
Brain	1.79 ± <0.01	1.79 ± 0.04	1.78 ± 0.01	1.74 ± 0.06
Ratio	0.79 ± 0.03	0.80 ± 0.03	0.81 ± 0.04	0.81 ± 0.05
Spleen	0.44 ± 0.04	0.45 ± 0.01	0.43 ± 0.01	0.45 ± 0.03
Ratio	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
Adrenals	0.04 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Ratio	0.02 ± 0.01	0.02 ± <0.01	0.02 ± <0.01	0.02 ± <0.01
Lungs	1.45 ± 0.14	1.34 ± 0.10	1.46 ± 0.07	1.09 ± 0.61
Ratio	0.64 ± 0.08	0.60 ± 0.05	0.66 ± 0.01	0.51 ± 0.29
Thymus	0.25 ± 0.04	0.22 ± 0.03	0.20 ± 0.03	0.19 ± 0.02
Ratio	0.11 ± 0.02	0.10 ± 0.01	0.09 ± 0.02	0.09 ± 0.1
Heart	0.79 ± 0.04	0.81 ± 0.05	0.81 ± 0.02	0.82 ± 0.06
Ratio	0.35 ± 0.01	0.36 ± 0.01	0.37 ± 0.02	0.38 ± 0.02

[a]Mean ± SD, N=5.
[b]Organ weight/body weight x 100.

Subchronic Inhalation Toxicity Study (28days)

Mean ± standard deviation concentrations of PBr_3 were 0.30 ± 0.03, 0.11 ± 0.01, and 0.034 ± 0.004 mg/L for target exposure concentrations of 0.3, 0.1, and 0.03 mg/L, respectively. Analytical to nominal concentration ratios for these exposures were 0.51, 0.50, and 0.62, respectively. The daily mean temperature for the exposure atmospheres ranged from 73.1 to 76.5 °F, and the daily mean relative humidity ranged from 2.4 to 3.6 %. No deaths occurred during the study. In addition, there were no treatment-related clinical signs observed in the study. Mean body weights for male and female rats in the control and PBr_3 exposure groups were similar (Tables 6 and 7).

Mean values of several serum chemistry and hematologic parameters were statistically significantly different in PBr_3 animals compared to those of the control group (Tables 8-11). Increases in serum chloride concentrations in rats of the 0.3 and 0.1 mg/L groups were attributable to interference with the presence of serum bromide (See Discussion section). A small increase in chloride values and a small decrease in ALT levels were observed in rats exposed to 0.3 mg/L PBr_3 (Tables 8 and 9). Though not concentration-related, an 11-26 % decrease in triglycerides was observed in all groups of rats exposed to PBr_3. Slight increases in mean corpuscular volume, monocytes, and eosinophils were observed in the high concentration exposed rats (Tables 10 and 11). A concentration-related decrease in platelets was observed in rats of the 0.3, 0.1, and 0.03 mg/L groups. Additional statistically significant differences in mean values of serum chemistry or hematologic parameters were sporadic and were not considered biologically significant.

No treatment-related gross lesions or differences in absolute or relative organ weights (Tables 12 and 13) were observed at necropsy.

Tissues from rats of the 0.3 mg/L PBr_3 and air-only control groups were examined microscopically. The only treatment-related finding was the presence of exudate or evidence of mild inflammation of the anterior nasal passages in 3 of 10 male rats and 1 of 10 female rats. This finding was not statistically significant compared to the control group values.

TABLE 6. BODY WEIGHTS[a] OF MALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Day of Study	Control	0.03 mg/L	0.1 mg/L	0.3 mg/L
-1	206 ± 7.9	206 ± 7.8	206 ± 8.7	205 ± 7.8
6	215 ± 8.4	215 ± 10.4	215 ± 8.6	215 ± 11.4
13	223 ± 7.2	224 ± 9.3	223 ± 9.8	220 ± 7.2
20	233 ± 7.1	232 ± 11.5	229 ± 9.3	227 ± 7.8
27	244 ± 8.8	244 ± 13.2	239 ± 10.5	236 ± 8.2

[a]Mean ± SD, N = 10.

TABLE 7. BODY WEIGHTS[a] OF FEMALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Day of Study	Control	0.03 mg/L	0.1 mg/L	0.3 mg/L
-1	137 ± 5.2	136 ± 5.8	136 ± 4.0	136 ± 5.4
6	147 ± 5.9	148 ± 5.5	147 ± 7.0	148 ± 8.8
13	147 ± 6.5	150 ± 5.5	149 ± 4.0	149 ± 8.5
20	150 ± 7.2	153 ± 6.2	150 ± 4.7	149 ± 8.2
27	154 ± 7.8	157 ± 6.6	154 ± 4.0	152 ± 9.7

[a]Mean ± SD, N = 10.

TABLE 8. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR MALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Parameter	Unit	Control	0.03 mg/L	0.1 mg/L	0.3 mg/L
BUN	(mg/dL)	15.7 ± 2.3	14.8 ± 1.9	16.3 ± 2.5	14.6 ± 1.8
Creatinine	(mg/dL)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Chloride	(mmol/L)	100.5 ± 1.1	103.1 ± 0.9	109.8 ± 2.2[c]	132.1 ± 5.4 [c]
Calcium	(mmol/L)	11.8 ± 0.3	11.8 ± 0.3	11.9 ± 0.2	12.2 ± 0.2[c]
Phosphorus	(mg/dL)	11.2 ± 0.5	11.5 ± 0.3	10.9 ± 0.4	11.4 ± 0.6
Total Protein	(g/dL)	6.5 ± 0.2	6.5 ± 0.3	6.5 ± 0.3	6.3 ± 0.3
AST	(IU/L)	75.7 ± 7.2	82.5 ± 8.0	78.5 ± 6.2	77.8 ± 7.5
ALT	(IU/L)	62.9 ± 8.8	61.1 ± 5.3	57.6 ± 8.6	47.8 ± 3.7[c]
Alkaline Phosphatase	(IU/L)	184.5 ± 19.1	181.9 ± 18.3	186.5 ± 22.7	188.5 ± 27.1
Glucose	(mg/dL)	177.3 ± 15.6	167.7 ± 32.6	169.6 ± 19.5	177.3 ± 14.2
Sodium	(mmol/L)	151.0 ± 1.2	150.5 ± 1.3	150.1 ± 1.5	150.0 ± 0.8
Triglycerides	(mg/dL)	100.4 ± 15.5	74.5 ± 31.0[c]	84.1 ± 24.5[c]	73.5 ± 12.4[c]
Magnesium	(mg/dL)	2.6 ± 0.3	2.7 ± 0.2	2.7 ± 0.1	2.7 ± 0.2
Potassium	(mmol/L)	4.6 ± 0.3	5.0 ± 0.4[b]	4.8 ± 0.3	4.9 ± 0.2
Cholesterol	(mg/dL)	55.0 ± <0.1	51.0 ± <0.1	55.0 ± <0.1	58.0 ± <0.1
Total Bilirubin	(mg/dL)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
ALB/GLOB	(g/dL)	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
CO_2	(IU/L)	36.7 ± 2.2	36.6 ± 1.6	36.7 ± 1.6	36.6 ± 1.6
Uric Acid	(mg/dL)	1.5 ± 0.3	1.6 ± 0.3	1.5 ± 0.3	1.5 ± 0.2
Albumin	(g/dL)	3.7 ± 0.2	3.6 ± 0.3	3.6 ± 0.2	3.5 ± 0.1
Globulin	(g/dL)	2.8 ± 0.1	2.9 ± 0.2	2.9 ± 0.2	2.8 ± 0.2
Creatine Kinase	(IU/L)	39.8 ± 10.1	43.7 ± 8.3	39.4 ± 11.5	40.5 ± 16.6
LDH	(IU/L)	207.0 ± 40.9	207.3 ± 26.3	238.0 ± 50.0	207.2 ± 70.0

[a]Mean ± SD, N = 10.
[b]Significantly different than control at $p<0.05$.
[c]Significantly different than control at $p<0.01$.

TABLE 9. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR FEMALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Parameter	Unit	Control	0.03 mg/L	0.1 mg/L	0.3 mg/L
BUN	(mg/dL)	18.6 ± 2.0	16.6 ± 2.5	18.1 ± 2.3	16.3 ± 1.5
Creatinine	(mg/dL)	0.5 ± <0.1	0.5 ± 0.1	0.5 ±<0.1	0.5 ± <0.1
Chloride	(mmol/L)	104.8 ± 2.0	106.4 ± 1.6	119.4 ± 2.2[d]	151.1 ± 2.9[d]
Calcium	(mmol/L)	11.2 ± 0.3	11.3 ± 0.2	11.4 ± 0.3	12.0 ± 0.2[d]
Phosphorus	(mg/dL)	9.7 ± 1.0	10.1 ± 1.0	10.4 ± 0.8	10.6 ± 0.5
Total Protein	(g/dL)	6.2 ± 0.2	6.0 ± 0.2	5.8 ± 0.2	6.1 ± 0.2
AST	(IU/L)	69.0 ± 7.3	70.8 ± 5.6	77.2 ± 10.8	75.9 ± 5.1
ALT	(IU/L)	50.4 ± 6.6	49.5 ± 9.2	55.7 ± 10.4	38.2 ± 10.5[d]
Alkaline Phosphatase	(IU/L)	169.9 ± 24.2	148.8 ± 15.1	165.8 ± 19.8	172.4 ± 28.7
Glucose	(mg/dL)	139.2 ± 14.7	126.5 ± 25.9	127.2 ± 24.7	129.5 ± 28.0
Sodium	(mmol/L)	149.7 ± 2.2	148.7 ± 1.2	148.3 ± 0.8	149.0 ± 0.9
Triglycerides	(mg/dL)	64.0 ± 13.2	57.1 ± 13.5[d]	47.4 ± 11.9[d]	48.5 ± 6.8[d]
Magnesium	(mg/dL)	2.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	2.5 ± 0.3
Potassium	(mmol/L)	4.9 ± 0.2	5.1 ± 0.4[c]	5.1 ± 0.4	5.1 ± 0.6
Cholesterol	(mg/dL)	78.1 ± 5.6	73.6 ± 5.6	71.6 ± 9.7	68.4 ± 8.8
Total Bilirubin	(mg/dL)	0.4 ± 0.1	0.4 ± <0.1	0.3 ± 0.1	0.4 ± 0.1
ALB/GLOB	(g/dL)	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
CO_2	(IU/L)	32.4 ± 1.9	33.5 ± 1.5	33.8 ± 1.2	34.0 ± 1.2
Uric Acid	(mg/dL)	1.1 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.4
Albumin	(g/dL)	3.4 ± 0.2	3.3 ± 0.1	3.2 ± 0.2	3.2 ± 0.1
Globulin	(g/dL)	2.8 ± 0.1	2.7 ± 0.2	2.6 ± 0.1	2.8 ± 0.2
Creatine Kinase	(IU/L)	48.4 ± 16.0[b]	41.4 ± 12.3	47.5 ± 18.5	50.5 ± 20.9
LDH	(IU/L)	323.6 ± 161.7	288.4 ± 69.1	289.7 ± 141.2	286.8 ± 113.4

[a]Mean ± SD, N = 10, [b]N = 9.
[c]Significantly different than control at p<0.05.
[d]Significantly different than control at p<0.01.

TABLE 10. BLOOD HEMATOLOGY VALUES[a] FOR MALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Parameter	Unit	Control	0.03 mg/L	0.1 mg/L	0.3 mg/L
WBC	(10^3)	9.9 ± 1.1	9.4 ± 1.4	8.9 ± 1.4	10.6 ± 1.3
RBC	(10^6)	9.6 ± 0.2	9.5 ± 0.3	9.6 ± 0.2	9.6 ± 0.3
HGB	(g/dL)	16.0 ± 0.7	16.0 ± 0.6	16.1 ± 0.8	16.3 ± 0.5
HCT	(%)	50.8 ± 1.0	50.8 ± 1.2	51.3 ± 0.8	51.5 ± 1.2
MCV	(g/dL)	53.2 ± 0.7	53.4 ± 0.5	53.5 ± 0.7	53.6 ± 0.8[b]
MCH	(g/dL)	16.7 ± 0.8	16.8 ± 0.9	16.8 ± 0.7	17.0 ± 0.4
MCHC	(g/dL)	31.4 ± 1.3	31.5 ± 1.5	31.3 ± 1.6	31.7 ± 0.7
Platelets	(10^3)	970.8 ± 55.3	954.5 ± 66.2[b]	905.8 ± 60.6[b]	875.7 ± 86.1[b]
Neutrophils	(%)	19.6 ± 2.6	18.8 ± 2.9	19.6 ± 2.8	18.7 ± 1.9
Lymphocytes	(%)	76.1 ± 3.2	75.7 ± 4.4	75.3 ± 4.3	75.4 ± 3.1
Monocytes	(%)	3.4 ± 2.5	4.6 ± 2.1	4.2 ± 2.1	4.9 ± 2.0[b]
Eosinophils	(%)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.1[b]
Basophils	(%)	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

[a]Mean ± SD, N = 10.
[b]Significantly different than control at $p<0.01$.

TABLE 11. BLOOD HEMATOLOGY VALUES[a] FOR FEMALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Parameter	Unit	Control	0.03 mg/L	0.1 mg/L[b]	0.3 mg/L
WBC	(10^3)	8.9 ± 1.1	7.8 ± 1.3	8.7 ± 1.1	10.1 ± 1.7
RBC	(10^6)	8.9 ± 0.2	8.9 ± 0.1	8.9 ± 0.2	8.8 ± 0.2
HGB	(g/dL)	15.5 ± 0.5	15.5 ± 0.3	15.6 ± 0.6	15.8 ± 0.4
HCT	(%)	48.8 ± 1.3	48.9 ± 0.6	49.2 ± 1.0	49.1 ± 0.9
MCV	(g/dL)	54.9 ± 0.5	55.1 ± 0.5	55.2 ± 0.4	55.6 ± 0.3[c]
MCH	(g/dL)	17.4 ± 0.4	17.4 ± 0.4	17.5 ± 0.8	17.9 ± 0.5
MCHC	(g/dL)	31.7 ± 0.8	31.6 ± 0.7	31.8 ± 1.3	32.2 ± 0.9
Platelets	(10^3)	987.7 ± 71.6	975.0 ± 43.0[c]	935.0 ± 67.4[c]	940.1 ± 62.0[c]
Neutrophils	(%)	17.6 ± 3.1	18.0 ± 3.6	17.3 ± 3.0	17.7 ± 3.0
Lymphocytes	(%)	79.0 ± 3.7	76.4 ± 4.7	76.7 ± 3.6	74.7 ± 4.2
Monocytes	(%)	2.6 ± 1.8	4.5 ± 1.5	5.0 ± 0.8	6.4 ± 1.4[c]
Eosinophils	(%)	0.8 ± 0.2	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2[c]
Basophils	(%)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± <0.1

[a]Mean ± SD, N = 10, [b]N = 9.
[c]Significantly different than control at $p<0.01$.

TABLE 12. ABSOLUTE ORGAN WEIGHTS[a] AND ORGAN-TO-BODY WEIGHT RATIOS OF MALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Organ	Control	0.03 mg/L	0.1 mg/L	0.3 mg/L
Body Wt.	228 ± 7.1	228 ± 10.2	221 ± 11.0	221 ± 7.3
Liver	7.04 ± 0.37	6.80 ± 0.45	6.70 ± 0.54	6.80 ± 0.39
Ratio	3.09 ± 0.11	2.98 ± 0.12	3.03 ± 0.15	3.08 ± 0.11
Kidneys	1.67 ± 0.10	1.68 ± 0.11	1.67 ± 0.11	1.67 ± 0.07
Ratio	0.73 ± 0.03	0.74 ± 0.05	0.76 ± 0.04	0.76 ± 0.03
Testes	2.68 ± 0.13	2.61 ± 0.19	2.24 ± 0.68	2.45 ± 0.19
Ratio	1.18 ± 0.07	1.15 ± 0.09	1.02 ± 0.31	1.11 ± 0.08
Brain	1.76 ± 0.04	1.78 ± 0.10	1.77 ± 0.06	1.74 ± 0.03
Ratio	0.77 ± 0.03	0.78 ± 0.05	0.80 ± 0.04	0.79 ± 0.02
Spleen	0.50 ± 0.02	0.51 ± 0.04	0.48 ± 0.03	0.47 ± 0.03
Ratio	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.02	0.21 ± 0.01
Adrenals	0.07 ± 0.03	0.20 ± 0.47	0.06 ± 0.01	0.05 ± 0.01
Ratio	0.03 ± 0.01	0.09 ± 0.20	0.03 ± 0.01	0.02 ± <0.0
Lungs	1.53 ± 0.13	1.51 ± 0.18	1.48 ± 0.12	1.56 ± 0.16
Ratio	0.67 ± 0.05	0.66 ± 0.08	0.67 ± 0.05	0.70 ± 0.07
Thymus	0.26 ± 0.02	0.25 ± 0.06	0.25 ± 0.06	0.22 ± 0.02
Ratio	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.02	0.10 ± 0.01
Heart	0.80 ± 0.06	0.79 ± 0.10	0.78 ± 0.04	0.78 ± 0.06
Ratio	0.35 ± 0.03	0.34 ± 0.04	0.35 ± 0.01	0.35 ± 0.02

[a]Mean ± SD, N = 10.

TABLE 13. ABSOLUTE ORGAN WEIGHTS[a] AND ORGAN-TO-BODY WEIGHT RATIOS OF FEMALE RATS EXPOSED FOR 28 DAYS TO PHOSPHORUS TRIBROMIDE VIA NOSE-ONLY INHALATION

Organ	CONTROL	0.03 mg/L	0.1 mg/L	0.3 mg/L
Body Wt.	143 ± 7.9	146 ± 5.9	143 ± 4.0	142 ± 10.8
Liver	3.89 ± 0.23	3.95 ± 0.26	3.88 ± 0.18	3.93 ± 0.35
Ratio	2.71 ± 0.08	2.70 ± 0.15	2.72 ± 0.11	2.76 ± 0.10
Kidneys	1.10 ± 0.11	1.10 ± 0.07	1.10 ± 0.04	1.12 ± 0.08
Ratio	0.77 ± 0.06	0.75 ± 0.04	0.77 ± 0.02	0.79 ± 0.02
Ovaries	0.08 ± 0.02	0.07 ± 0.02	0.11 ± 0.08	0.09 ± 0.05
Ratio	0.05 ± 0.02	0.05 ± 0.01	0.07 ± 0.06	0.06 ± 0.03
Brain	1.64 ± 0.06	1.65 ± 0.05	1.66 ± 0.04	1.63 ± 0.09
Ratio	1.15 ± 0.06	1.13 ± 0.04	1.17 ± 0.04	1.14 ± 0.03
Spleen	0.34 ± 0.02	0.36 ± 0.02	0.36 ± 0.02	0.37 ± 0.03
Ratio	0.24 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01[b]
Adrenals	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.01	0.07 ± 0.05
Ratio	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.03
Lungs	1.14 ± 0.09	1.14 ± 0.04	1.15 ± 0.09	1.14 ± 0.14
Ratio	0.79 ± 0.04	0.78 ± 0.04	0.81 ± 0.05	0.80 ± 0.07
Thymus	0.19 ± 0.04	0.20 ± 0.03	0.20 ± 0.02	0.17 ± 0.04
Ratio	0.13 ± 0.03	0.14 ± 0.02	0.14 ± 0.01	0.12 ± 0.02
Heart	0.55 ± 0.07	0.55 ± 0.05	0.54 ± 0.02	0.55 ± 0.03
Ratio	0.39 ± 0.04	0.37 ± 0.03	0.38 ± 0.02	0.39 ± 0.04

[a]Mean ± SD, N = 10.
[b]Significantly different than Control at $p<0.01$.

SECTION IV

DISCUSSION

Application of neat PBr_3 causes an immediate reaction that results in edema and necrosis of the skin. Microscopic examination of the treated skin indicates necrosis through the epidermis and dermis, the panniculus muscle, and into the skeletal muscle of the underlying subcutis. Rinsing with water 30 seconds after application of neat PBr_3 did not alter the severity of the effect on the skin, though the extent of damage was not observed in the underlying subcutis. The appearance of these lesions were considered comparable with an acute chemical injury (burn).

To administer PBr_3 vapor to animals, special conditions were set-up. Relative humidity was removed from the air entering the exposure chambers to limit as much as possible the reaction of PBr_3 with water vapor. For safety considerations, nose-only inhalation exposures were conducted. This effort limited the total amount of PBr_3 vapor that was required for the inhalation studies and confined the work area due to the small size of nose-only chambers compared to whole-body chambers. To monitor the concentration of PBr_3 vapor during animal exposures, an analytical method was developed that measured bromide ion following the reaction of PBr_3 vapor with water. Details of the analytical procedure are given in Appendix A.

Acute 4-h nose-only exposure of Fischer 344 rats to PBr_3 vapor resulted in mortality at 4.1 mg/L. At 1.5 mg/L, labored breathing, body weight loss, ulceration of anterior nares, and histopathologic lesions (rhinitis) of the nasal passage were observed. Signs of toxicity were also observed at 0.9 mg/L PBr_3. Clinical signs, gross lesions, and microscopic tissue lesions were absent in rats exposed for 4 h to 0.4 mg/L PBr_3. This series of acute animal exposures provides good concentration-response data for single, high PBr_3 concentration exposure scenarios.

In the 5-day concentration range-finding study, there were no overt signs of toxic stress. Rats of the 0.51 mg/L group had a statistically significant decrease in body weight means compared to the controls. Gross lesions (reddened nares) and microscopic lesions (suppurative inflammation of mucosa and ulceration of epithelium of the external nares) were observed in rats of the 0.51 mg/L group. Slight inflammation of the nasal mucosa (most anterior regions) was also observed in a rat of the 0.16 mg/L group. Rats in the 0.06 mg/L group were without adverse effects, and values of measured parameters were similar to those

of the air-only control rats. These results were used to select target exposure concentrations for the 28-day inhalation study. The target concentrations were 0, 0.03, 0.1, and 0.3 mg/L PBr_3.

In the 28-day study, there were no signs of toxic stress, alterations in body weights, or changes in organ weights in PBr_3 exposed animals. Minor serum chemistry and hematology effects were observed in the treated animals. Microscopic tissue findings were limited to rats of the 0.3 mg/L group and consisted of mild inflammation of the anterior nasal passages.

Note that a decrease in mean body weight was observed in all exposure groups, including the control group, in both the 5-day and 28-day inhalation studies. Exposure systems requiring animal restraint may cause animal stress (review by Phalen et al., 1984), such as excessive heat, decreases in body weight, or alterations in pulmonary function. Note, also, the increase in serum chloride concentrations in PBr_3 exposed rats only in both the 5-day and 28-day studies. The increase in serum chloride levels in PBr_3 exposed rats only is believed to be an artifact, due to interference from ionized bromide in the test material with the serum chloride assay. Information collected from the procedure manual for the chloride assay (Vitros CAT No. 8120446, 11/96) indicates bromide and iodide (from therapeutic drugs) are known interfering substances.

On the basis of results observed in these studies, neat PBr_3 is corrosive when applied to the skin, the acute (4-h) lethal concentration of PBr_3 is approximately 4 mg/L, and a concentration of 0.1 mg/L is the no observable adverse effect level (NOAEL) in the 28-day inhalation study.

SECTION V

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SECTION VII

APPENDIX A

Phosphorus Tribromide (PBr_3) Analytical Report - Methods

The test material phosphorous tribromide (PBr_3) reacts rapidly with water vapor present in air forming phosphorous acid and hydrogen bromide. It is also a good brominating agent. These two properties negated the use of our usual methods for exposure concentration analysis, IR absorbance or gas chromatography. The next approach was to make use of its water reactivity to free the bromide ion for analysis, then to use a bromide combination specific ion electrode (ATI Orion, Boston, Ma 02129) to determine the bromide concentration. Quantitative addition of PBr_3 directly to water validated this approach as the preferred method for analysis. Further, in testing for the efficiency of absorption of PBr_3 and the formation the bromide ion, a set of three bublers in series demonstrated that >95% was absorbed in the first tube.

The rapid reactivity and sample line loss negated a continuous analysis system. A direct method for analysis was chosen for confirming the nominal concentration by spot sampling every 20 minutes. During the acute portion of the study, 8 mL of ionic strength adjusted water (94 Series Ionic Strength Adjuster, ATI Orion, Boston, Ma 02129) was used to absorb and react with the PBr_3 from an 80 mL air sample in a 100 mL syringe. The bromide specific ion electrode, calibrated with known bromide ion concentration standards (ATI Orion, Boston, Ma 02129) was used to determine the concentration of the samples thus allowing calculation of the vapor concentration. For lower concentration analysis the ratios of sample to absorber was increased.

28-Day Study

Phosphorous tribromide (PBr_3) is highly reactive and must be handled in the hood with double glove protection. To prevent decomposition of the PBr_3 test substance after filling glass syringes, the headspace of the PBr_3 stock container was filled with dry nitrogen before closing.

Due to the potential for overrun when starting Sage syringe pumps, it was necessary that the animal breathing air be clean until the start of the exposure and the initial start-up high

concentration bypassed the Cannon-52 system. The same clean air flow could be reestablished in the event of a pump malfunction.

Exposure Routine

1. Have the animals in the Cannon-52 operating with clean air before filling the syringes.
2. Fill the syringes to about 200% of the days needed supply.
3. Start the syringes in waste operation mode as soon as filled so that any excess at the start bypasses the animals. (High 1.0 mL, full syringe; Mid 0.5 mL, full syringe; Low > 0.2 mL near full).
4. Switch to exposure operation conditions as ready.
5. Read the generation supply syringe position in cm as monitor for input rate and nominal calculations every 20 minutes.
6. Enter the data in the operation file as soon as possible to keep aware of the nominal.
7. Draw the samples for ion analysis using the 60 mL plastic syringes. Use 5 mL of water containing 2% Ionic Strength Buffer Titrated to pH of 4. 50 mL air sample for the high chamber; two 50 mL samples for the mid, and four 50 mL samples for the Low chamber. (Multiple samples are drawn, shaken to dissolve the contaminant, then the air expelled to allow the next intake).
8. The Bromide probe needs calibration daily before star-up and also at least once during the days run. If any thing regarding the analysis appeared not working properly, this calibration was rechecked.
9. At termination of the exposure, the air was switched for clean to animals, and generation line, to waste.
10. Also, at termination the evaporation zone must be observed for accumulation of any breakdown materials or condensates, cleaned and dried before the next days operation.

OPERATION PARAMETERS

High chamber Target 0.3 mg/L Use a 1 mL syringe, fill to 1 mL.

Sage (AMRL 5757)

Set range 1000 and ~100% adjust as needed.

Mid Chamber Target 0.1 mg/L Use a 0.5 mL syringe, fill to 0.5 mL.

Sage (AMRL 5753)

Set range 1000 and ~50% adjust as needed.

Low Chamber Target 0.03 mg/L Use a 0.25 mL syringe, fill to 0.25 mL.

Sage (AMRL 5754)

Set range 1000 and ~40% adjust as needed.

Bromide Analysis

Use the syringe method for analysis. Use a separate syringe for each concentration and do a double* absorption for the mid chamber sample and 4X50 ml for the low chamber. Soak the probe in buffer** at a concentration near that of the expected mid range level sample. Make appropriate range of standards for the exposure set. (Need to bracket low and high values.) Dilute the standard 0.1M Sodium bromide from Orion to 1μM in decade values. If the low end of the curve is non-linear with the log concentration, make standards between the decade vaues (as 2, and 4.5 μM, and 20 and 45 μM.) Calculate the PBr_3 concentration from the Br– ion concentration. 90 + % of samples were between 10 and 100μm.

*Fill syringe with 5 mL absorber, 50 mL sample, shake and expel the air, take the next 50 mL sample of air.

**Buffer 94 Series Ionic Strength adjuster, ATI Orion (pH to 4.0 for solution)